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Non-steady-state processes in amperometric biosensors: modelling studies

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Abstract. The non-steady-state processes of a biosensor based on a diffusion-limited oxygen sensor were investigated. The effect of various processes occurring in the biosensor on its overall response was studied. A mathematical model of transient processes of the biosensor was elaborated. The maximum rate of current change was taken as a measure of sensor response to substrate concentration. The validity of the model was tested by using a biosensor with immobilized microorganisms.

Key words: microbial biosensor, agarose-gel film, mathematical modelling, non-steady-state process, dynamic method.

INTRODUCTION

The biosensor is defined as a self-contained integrated device capable of providing specific quantitative analytical information using a biological recognition element [1]. Enzymes and whole cells are commonly used as biological material in amperometric biosensors. Biochemical oxygen demand (BOD) biosensors have been developed to measure BOD faster than by the conventional method (BOD_5), which requires a 5 day incubation of the solution. These sensors contain a microbial membrane in intimate contact with an oxygen sensor and allow the determination of a wide range of substances.

Most frequently the biosensor output signal is analysed according to the stabilized initial and final values. It was shown that the total oxygen uptake measurement needs 15–20 min and the recovery time between measurements can be as long as 3–4 h [2]. An alternative approach to the steady-state analysis of a biosensor's response [3–5] is through the mathematical modelling of the

dynamic behaviour of the output values. Several models have been developed to describe the steady-state and transient response of biosensors of different designs and modes of operation [6–9].

The purpose of this study was to develop a mathematical model describing the transient processes of biosensors. The dynamic method of measurement based on the determination of the maximum rate of current change during the experiment allows the researchers to carry out faster measurements in a wider substrate concentration range compared to steady-state output of a biosensor.

THEORETICAL MODEL OF A BIOSENSOR

The amperometric biosensor used in this study is based on a diffusion-limited amperometric oxygen sensor (the Clark-type oxygen sensor [10]) with an additional gel membrane containing immobilized microorganisms. The substrate is metabolized by microorganisms consuming oxygen in the microbial membrane of the sensor. This process leads to the redistribution of oxygen fluxes. As a result, the concentration of oxygen decreases at oxygen sensor's membrane "m" leading to a decrease in the biosensor output current. A schematic diagram of the biosensor and the fluxes of oxygen and substrate in sensor layers is depicted in Fig. 1.

The non-steady-state response of the biosensor is observed through a dynamic change in substrate concentration. A step change in substrate concentration is used in order to investigate transient processes in a biosensor. The concentration

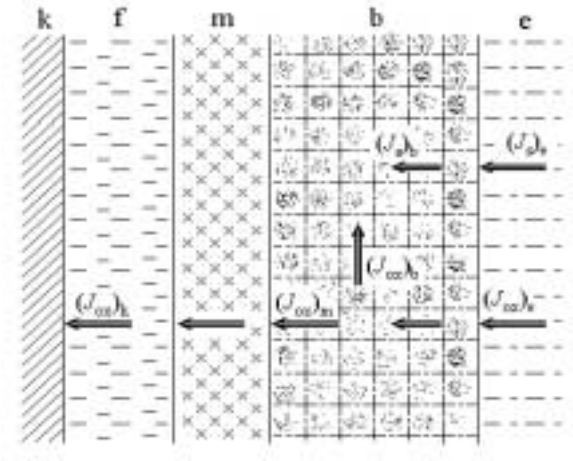

Fig. 1. Schematic diagram of biosensor layers: k, sensor's cathode; f, electrolyte film; m, membrane of oxygen sensor; b, gel layer with immobilized microorganisms; e, test medium; $(J_{ox})_k$, flux of oxygen towards the sensor's cathode; $(J_{ox})_m$, flux of oxygen at the surface of the membrane; $(J_s)_b$, flux of substrate degraded by microorganisms; $(J_{ox})_b$, flux of oxygen consumed by microorganisms; $(J_s)_e$, flux of substrate coming from the test medium; $(J_{ox})_e$, flux of oxygen from the test medium.

of oxygen (C_{mb}) at the sensor's membrane "m" can be assumed to be an exponential function of time (see Fig. 2a). The time lag at the beginning of the curve is caused by the transient processes taking place in the layer containing microorganisms. The amplitude and time constant of the exponent depend on the magnitude of the step change of substrate concentration and on the diffusion layer parameters for O_2 diffusion.

The change in the oxygen concentration at membrane "m" causes a corresponding change in the oxygen flux $(J_{ox})_k$ towards the cathode (Fig. 2b). The flux $(J_{ox})_k$ is directly proportional to the sensor current.

For an exponential form of the dependence of C_{mb} on t the sensor's current I as a function of time can be expressed by the following equation [11]:

$$I(t) = I_\infty + \Delta I \times h^*(t), \tag{1}$$

where $h^*(t)$ is the transfer function of the sensor; $\Delta I = I_0 - I_\infty$ is the change in $I = f(t)$ in a time interval between 0 and ∞; I_0 and I_∞ are the initial and final values of the current, respectively.

The transfer function of the sensor is expressed by:

$$h^*(t) = \exp\left(-\frac{t}{T_s}\right) - 2\sum_{n=1}^{\infty}(-1)^n \times \frac{T_d}{n^2 T_s - T_d}\left[\exp\left(-\frac{t}{T_s}\right) - \exp\left(-n^2\frac{t}{T_d}\right)\right], \tag{2}$$

where T_d and T_s are the time constants for the oxygen sensor and for the exponential function of $C_{mb} = f(t)$, respectively.

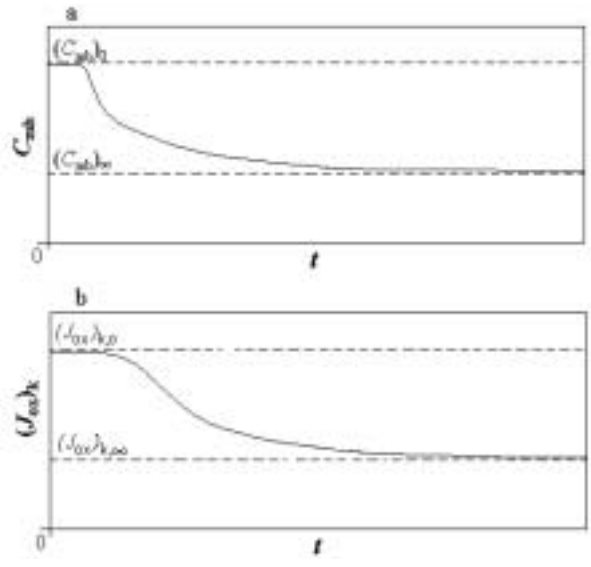

Fig. 2. Transient processes in oxygen sensor: a, time dependence of oxygen concentration at the outer surface of O_2 sensor membrane; b, time dependence of the flux of oxygen towards the sensor's cathode.

Figure 3 presents the time dependence of the sensor current by a step change in substrate concentration. The transfer function $h^*(t)$ of the oxygen sensor is characterized by its inflection point "p" at which the rate of change of the transfer function $(\mathrm{d}h^*/\mathrm{d}t)$ shows a maximum value [12] and, therefore, the function of the current on time $(I = f(t))$ also shows an inflection point at which the rate of change of the current $(\mathrm{d}I/\mathrm{d}t)$ is at maximum. This maximum rate of current change is very convenient for experimental investigation. Taking into consideration that $\delta I = \Delta I / I_0$, the equation for the maximum rate of the change of the sensor current can be obtained by the differentiation of Eq. 1:

$$\left[\frac{\mathrm{d}I}{\mathrm{d}t}\right]_{\max} = I_0 \delta I \left[\frac{\mathrm{d}h^*}{\mathrm{d}t}\right]_{\max}, \tag{3}$$

where $(\mathrm{d}h^*/\mathrm{d}t)_{\max}$ is the maximum value of $\mathrm{d}h^*/\mathrm{d}t$.

The normalized output current of the biosensor is a function of substrate concentration in the test medium [13]

$$\delta I = \frac{\delta I_{\max} C_{\mathrm{se}}}{K_{\mathrm{m}} + C_{\mathrm{se}}}, \tag{4}$$

where $\delta I_{\max}$ is the maximum value of δI, C_{se} is the concentration of the substrate, and K_{m} is the half-saturation constant.

Representative curves of the dependence of δI on C_{se} are shown in Fig. 4. It should be mentioned that the dependence of δI on substrate concentration $(\delta I = f(C_{\mathrm{se}}))$ is a steady-state function by its physical origin because it is determined only by the initial (I_0) and final (I_∞) values of the current.

For $\delta I_{\max} > 1$ the function $\delta I = f(C_{\mathrm{se}})$ acquires its limiting value at $\delta I = 1$ (see Fig. 4, curve a). The limiting point of the function $(\delta I = f(C_{\mathrm{se}}))$ corresponds to a substrate concentration at which all the oxygen is consumed in

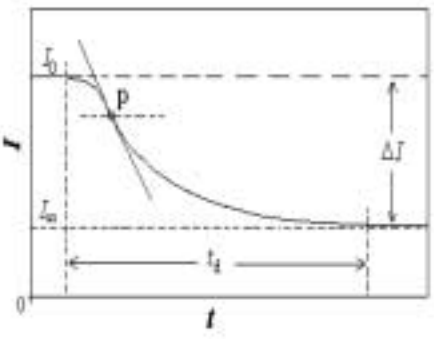

Fig. 3. Sensor current as a function of time by a step change in substrate concentration. t_{d}, time of transient process; p, inflection point.

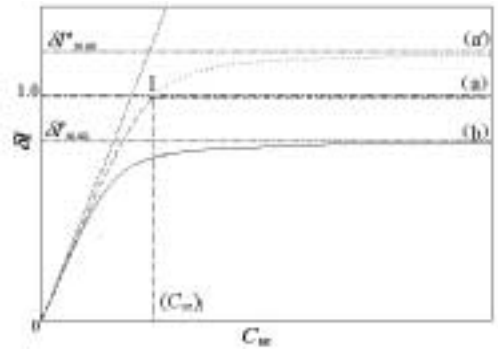

Fig. 4. Dependence of δI on C_{se}. Curve (a) corresponds to the case of $\delta I_{max} > 1$, (a′) is the hypothetical curve in the absence of oxygen concentration limit, and curve (b) represents a typical dependence of δI on C_{se} for $\delta I_{max} < 1$.

a sensor layer containing a biological component and the sensor current drops to zero level. The hypothetical curve a′ in Fig. 4 would correspond to a case with no limitation by oxygen concentration. For $\delta I_{max} < 1$ the function $\delta I = f(C_{se})$ is not limited by $\delta I = 1$ (see Fig. 4, curve b). The maximum rate of current change as a function of substrate concentration can be obtained by combining Eqs. 3 and 4:

$$\left(\frac{\mathrm{d}I}{\mathrm{d}t}\right)_{max} = I_0 \times \frac{\delta I_{max} C_{se}}{K_m + C_{se}} \times \left(\frac{\mathrm{d}h^*}{\mathrm{d}t}\right)_{max} . \tag{5}$$

For experimental investigation the biosensor was connected with an electronic device. The output signal can be given in the units of concentration as a direct proportionality exists between the concentration of oxygen and the sensor current ($C_\alpha = k_\alpha \times I$). Therefore, by substituting δC_α for δI in Eq. 4 the following equation can be derived:

$$\delta C_\alpha = \frac{\delta C_{\alpha,max} C_{se}}{K_m + C_{se}}, \tag{6}$$

where $\delta C_{\alpha,max} = \delta I_{max}$. Analogously, the transformation of Eq. 5 yields

$$\left(\frac{\mathrm{d}C_\alpha}{\mathrm{d}t}\right)_{max} = C_{\alpha,0} \times \frac{\delta C_{\alpha,max} C_{se}}{K_m + C_{se}} \times \left(\frac{\mathrm{d}h^*}{\mathrm{d}t}\right)_{max}, \tag{7}$$

where $(\mathrm{d}C_\alpha/\mathrm{d}t)_{max}$ is the maximum rate of change of oxygen concentration.

EXPERIMENTAL

The amperometric biosensor based on the oxygen-measuring principle consists of two main parts: an oxygen sensor and a microbial agarose-gel membrane. The dissolved oxygen sensor Cellox325 (WTW, Germany) was used. A specially designed holder was used to attach an agarose-gel membrane with immobilized microorganisms to the oxygen probe as described previously [13].

The microorganisms used were isolated from baker's yeast, suspended in phosphate buffer (pH 6.8), and immobilized on the agarose-gel membrane. The microbial membrane was made of the 2% solution of agarose in phosphate buffer and of the suspension of the microorganisms. The agarose solution heated to the boil was cooled down to 45 °C, and 2 mL of yeast suspension was added to it. The resulting mixture was spread on the polymer net of a particular thickness which, in order to gain a certain and even thickness of the layer containing microorganisms, was then placed between two glass plates until the formation of a persistent layer of gel. The microbial membrane was kept for 5 days in glucose solution in phosphate buffer to reach a stable condition. The biosensor response was measured in glucose solutions in a concentration range of 25–1000 mg L^{-1}.

The measurements were carried out at 25 °C in a 100 mL thermostatted measuring cell. During the experiment the solutions were continuously mixed with a magnetic stirrer and saturated by air oxygen with a microcompressor. Glucose solution was added to the air-saturated test medium after the steady-state output of the biosensor had been obtained. The sensor output signal was registered in a time interval of 1 s.

RESULTS AND DISCUSSION

The biosensor signals were detected in the solutions of glucose of various concentrations for testing the mathematical model. The sensor's response was analysed according to the steady-state method and with the herein developed model of transfer processes.

Figure 5 presents the normalized biosensor response vs. time curves at various substrate concentrations. It is noticeable that at higher concentrations of glucose (curves 5 and 6 in Fig. 5) the response of the sensor drops to zero and δC_α gains a maximum value. The steady-state values of the output signal were used to construct the δC_α vs. C_{se} plot shown in Fig. 6. The solid line corresponding to the theoretical curve based on Eq. 6 was obtained by the least squares curve fitting. The theoretical curve exceeds the limiting value of unity at a certain substrate concentration $(C_{se})_1$ above which the biosensor becomes nonsensitive towards substrate concentration. However, the concentration range in which the substrate can be correctly determined with the given biosensor is even lower than $(C_{se})_1$. The maximum rate of change of the oxygen sensor output $((\mathrm{d}C_\alpha/\mathrm{d}t)_{max})$ derived from the experimental $C_\alpha/C_{\alpha,0}$ vs. t curves is

plotted against glucose concentration in Fig. 7. The theoretical curve for the dependence of $(\mathrm{d}C_\alpha/\mathrm{d}t)_{\max}$ on C_{se} was also obtained by using the method of least squares curve fitting (Fig. 7). Equation 7 was used as a basic equation for curve fitting. It is of great practical importance that we should be far from the limiting value in the corresponding $(\mathrm{d}C_\alpha/\mathrm{d}t)_{\max}$ vs. C_{se} curves in the concentration range under study.

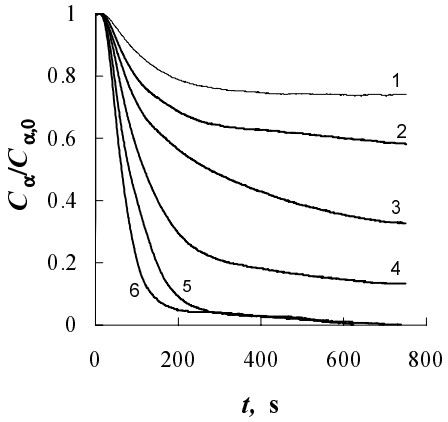

Fig. 5. Normalized response of a biosensor at various glucose concentrations: 1, 25 mg L^{-1}; 2, 115 mg L^{-1}; 3, 210 mg L^{-1}; 4, 380 mg L^{-1}; 5, 630 mg L^{-1}; 6, 930 mg L^{-1}.

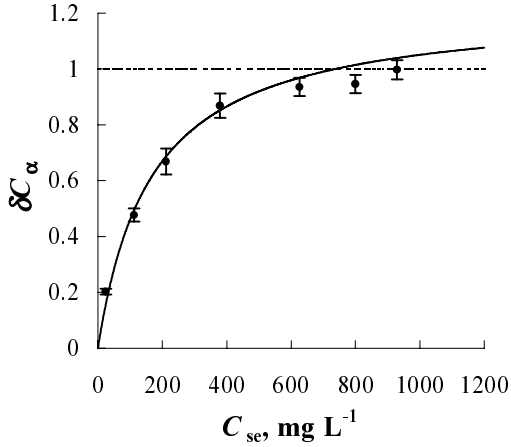

Fig. 6. Dependence of δC_α on glucose concentration for a biosensor. The solid line corresponds to the theoretical curve.

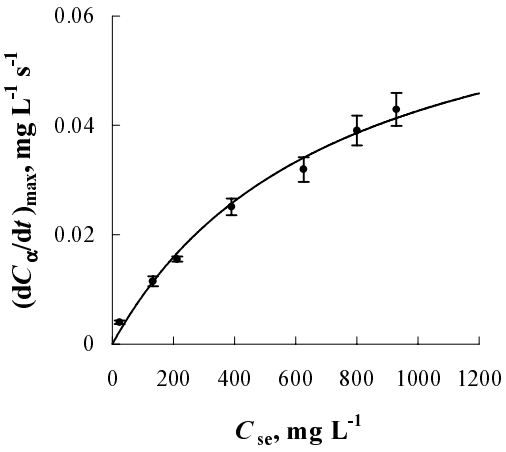

Fig. 7. Dependence of $(\mathrm{d}C_\alpha/\mathrm{d}t)_{\max}$ on glucose concentration for a biosensor. The solid line corresponds to the theoretical curve.

The use of the method of analysing the transfer processes of a biosensor response thus provides a much broader detectable concentration range and shorter response and regeneration times than steady-state output. On the basis of these observations it is obvious that the method of using the maximum rate of change is more advantageous than determination of stabilized initial and final values of the response for the practical application of biosensors.

CONCLUSIONS

The description of non-steady-state processes of an amperometric biosensor is presented as a mathematical model. The model of the biosensor enables us to define the form of functional dependences between the variable and constant quantities characterizing the processes. The most important of these dependences is the transfer function of the biosensor $(\mathrm{d}I/\mathrm{d}t)_{\max} = f(C_{se})$, which describes mathematically the dependence of the maximum rate of the current change on substrate concentration.

One of the main advantages of the method of using the transfer function of the biosensor in comparison with the dependence of δI vs. C_{se} lies in the fact that it allows us to widen the range of substrate concentration to be measured. It also enables the researcher to reduce the time of measurements. The developed theoretical model of the non-steady-state processes of a biosensor is an extension of the work on the mathematical modelling of steady-state processes [14].

LIST OF SYMBOLS

C_{mb} – concentration of oxygen at the outer surface of O_2 sensor membrane m
$(C_{mb})_0$ – value of C_{mb} at $t = 0$
$(C_{mb})_\infty$ – value of C_{mb} for $t \to \infty$
C_{se} – concentration of substrate in the test medium
$(C_{se})_l$ – value of C_{se} at the point of limit l
C_α – oxygen sensor output in the units of concentration
$C_{\alpha,0}$ – value of C_α at $t = 0$
$C_{\alpha,\infty}$ – value of C_α for $t \to \infty$
ΔC_α – total change of the value of C_α
δI_α – normalized oxygen sensor output
$\delta C_{\alpha,max}$ – maximum value of δC_α
dC_α/dt – rate of change of C_α
$(dC_\alpha/dt)_{max}$ – maximum value of dC_α/dt
$h^*(t)$ – transfer function of sensor
dh^*/dt – rate of change of transfer function $h^*(t)$
$(dh^*/dt)_{max}$ – maximum rate of change of transfer function
I – output current of biosensor
dI/dt – rate of change of output current
$(dI/dt)_{max}$ – maximum value of dI/dt
δI – normalized output current
δI_{max} – maximum value of the normalized output current
ΔI – total change of the value of the current
I_0 – biosensor's current at $t = 0$
I_∞ – biosensor's current for $t \to \infty$
$(J_{ox})_b$ – flux of oxygen consumed in microbial layer b
$(J_{ox})_e$ – flux of oxygen from solution bulk e
$(J_{ox})_k$ – flux of oxygen towards the sensor's cathode k
$(J_{ox})_{k,0}$ – flux of oxygen $(J_{ox})_k$ at $t = 0$
$(J_{ox})_{k,\infty}$ – flux of oxygen $(J_{ox})_k$ for $t \to \infty$
$(J_{ox})_m$ – flux of oxygen reaching membrane m
$(J_s)_b$ – flux of substrate consumed in microbial layer b
$(J_s)_e$ – flux of substrate from solution bulk e
K_m – half-saturation constant
k_α – coefficient of proportionality between I_k and C_α
t – time
t_d – time of the transient process
T_d – time constant of the sensor
T_s – time constant of the exponential function $C_{mb}(t)$

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Mittestatsionaarsete protsesside modelleerimine amperomeetrilises biosensoris

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On esitatud amperomeetrilise biosensori mittestatsionaarseid protsesse kirjeldav matemaatiline mudel. See võimaldab määrata protsessis muutuvate ja konstantsete suuruste omavahelisi sõltuvusi. Neist kõige tähtsam on biosensori üle-

kandefunktsioon $(\mathrm{d}I/\mathrm{d}t)_{\max} = f(C_{\mathrm{se}})$, mis kirjeldab matemaatiliselt sensori väljundvoolu muutuse maksimaalse kiiruse sõltuvust substraadi kontsentratsioonist.

Biosensori ülekandefunktsiooni kasutamine võimaldab suurendada substraadi kontsentratsiooni määramispiirkonda võrreldes sõltuvusega δI vs C_{se}. Ühtlasi väheneb eksperimendi läbiviimiseks vajalik aeg. Biosensori mittestatsionaarsete protsesside mudel on statsionaarsete protsesside matemaatilise mudeli edasiarendus.